

Enzymatic Browning Control in Potato with Ascorbic Acid-2-Phosphates

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ABSTRACT

Treated potato samples were evaluated for browning by tristimulus colorimetry and for browning inhibitor uptake by HPLC. Treatment effectiveness was greatly improved by reducing pH to 2.0 with phosphoric acid to inhibit endogenous acid phosphatase and by using combinations of ascorbic acid (AA), AA-2-phosphate and AA-2-triphosphate to provide for gradual release of AA at treated surfaces. Treatment with dips containing the AA-2-phosphates extended storage life of potato dice and pre-peeled potatoes by 5-7 days over that obtained with conventional browning inhibitor formulations but induced some leakage at cut surfaces.

Key Words: potato, browning, enzyme, phosphate, inhibitors

INTRODUCTION

THE STORAGE LIFE of peeled or cut raw potatoes is limited by the onset of enzymatic browning at cut surfaces. The discoloration results from the polyphenol oxidase-catalyzed oxidation of phenolic compounds to quinones and their subsequent condensation to dark-colored pigments (Matheis, 1986). In high pressure steam peeled potatoes, this defect may be accompanied by after-cooking darkening. This heat-induced reaction results in formation of a dark-colored complex of ferric ion and an *ortho*-dihydric phenol (Smith, 1987).

Previously, potato processors controlled browning in raw products by the application of sulfites, highly effective browning inhibitors (Feinberg et al., 1987). However, because of adverse health effects on some asthmatics, the use of sulfites for this purpose has been banned by the FDA (Anon., 1990). Various sulfite substitutes, generally combinations of ascorbic acid (AA) or erythorbic acid, with such adjuncts as citric acid (CA), calcium salts, sodium acid pyrophosphate (SAPP) or other phosphates, and cysteine, have been marketed (Duxbury, 1987, 1988). However, these products are less effective than sulfites, and treated products may require vacuum or modified atmosphere packaging (Langdon, 1987; O'Beirne and Ballantyne, 1987) or packing in a preservative cover solution (Santerre et al., 1991) to achieve the desired storage life of 14 days. Recently, Molnar-Perl and Friedman (1990) described the use of reduced glutathione and N-acetyl-L-cysteine as highly effective alternatives to sulfite for cut potatoes.

In previous studies, we demonstrated the efficacy of the AA-2-phosphates, stable, slow-release sources of AA, as browning inhibitors for apples (Sapers et al., 1989), but these derivatives did not successfully control browning in potatoes. This failure appeared to be a consequence of the high level of acid phosphatase (APase) activity in potato, compared to apple, which results in the rapid hydrolysis of AA-2-phosphates and premature consumption of the AA generated thereby (Sapers et al., 1990). Our objective in the present study was to develop browning inhibitors based on the AA-2-phosphates, that would overcome this limitation and would be more effective than AA-based formulations for cut and pre-peeled potatoes.

MATERIALS & METHODS

Raw materials and sample preparation

Russet Burbank potatoes were obtained from the Aroostook Experimental Farm in Presque Isle, ME and stored at 3°C until needed. Additional potatoes, designated as "Russet" or "round-white" types were obtained from local food stores. Potato plugs, about 2.2 cm in diameter, and 0.95 cm dice, to be treated with browning inhibitors, were prepared from washed tubers, as described previously (Sapers et al., 1990). Pre-peeled tubers were prepared by abrasion peeling with a Toledo Vegetable Peeler (Model A1-15; Toledo Scale Co., Toledo, OH) or high pressure steam peeling at 1400 kPa for 15.5 sec, followed by washing with a high pressure water spray. All peeled tubers were immediately immersed in a holding solution containing 2% sodium acid pyrophosphate (SAPP) and 0.25% NaCl to delay browning.

Application of browning inhibitors

Conventional browning inhibitor formulations comprised solutions of ascorbic acid (AA), citric acid (CA), SAPP, and CaCl₂, in some experiments adjusted to pH 2 or 3 with HCl or H₃PO₄. Experimental formulations contained those components in combination with ascorbic acid-2-phosphate (AAP) and/or ascorbic acid-2-triphosphate (AATP). Alternative formulations and controls were compared by measuring the extent of browning at treated potato surfaces in three systems: the tangentially cut surface of plugs, prepared with a sharp knife; the randomly oriented cut surfaces of a 50-g potato dice sample; and the peeled surface of plugs cut from pre-peeled potatoes. Treatments were applied to replicate plugs from replicate tubers by immersing the freshly prepared tangentially cut surface in browning inhibitor solution for 1.5 or 5 min, draining in a colander, blotting the plug circumferential area on absorbent tissue, and placing the treated samples in covered crystallizing dishes to avoid dehydration during storage (Sapers and Douglas, 1987). Potato dice samples (ca. 200g) were immersed in 200 mL browning inhibitor solution for 5 min, drained in a colander for about 1 min, and packaged in plastic bags. Pre-peeled potatoes were treated by cutting duplicate plugs from each of two identically treated peeled tubers, submerging the peeled surface in browning inhibitor solution, and then draining, blotting and packaging, as described. Control plugs were dipped in water for 20 sec to remove adhering juice and free starch, drained and blotted, similar to treated plugs.

All samples were stored at 4°C and evaluated at intervals by tristimulus colorimetry. Changes in the color of tangentially cut or pre-peeled surfaces of potato plugs were measured with a Gardner XL-23 tristimulus colorimeter (Byk-Gardner, Silver Spring, MD), standardized with a white tile ($L = 91.97$; $a = -1.43$; $b = 1.47$). Changes in the color of potato dice were determined on quadruplicate 50-g samples, placed in optical glass beakers, and measured with a Byk-Gardner spectrophotometer (The Color Machine), as described previously (Sapers et al., 1990). Changes in L - and a -values (ΔL and Δa) and values of the percent inhibition, a parameter used to compare treatments (Sapers and Douglas, 1987), were computed at each storage interval. Based on our previous visual observations and reflectance measurements of potatoes undergoing enzymatic browning, we considered a percent inhibition value of 50-60%, based on the change in a -value, to represent the limit of acceptability for raw potato (Sapers et al., 1990).

Determination of AA and AAP by HPLC

AA and AAP in treated Russet Burbank plugs were determined by HPLC on an aminopropyl bonded-phase silica column, eluted isocratically with acetonitrile-0.05M KH₂PO₄ (75:25), as described pre-

Table 1—Effects of pH and phosphate on acid phosphatase activity in Russet Burbank juice

| Juice pH | Activity ^a (Units/100g) |
|----------------------------|------------------------------------|
| without Phosphate | |
| 2.0 | 4 ± 1.8 |
| 3.0 | 61 ± 22.0 |
| 3.9 | 129 ± 0.2 |
| 4.7 | 221 ± 12.4 |
| 5.8 | 231 ± 5.2 |
| with Phosphate (mM) | |
| 3.0 | 24 ± 0.2 |
| 50 | 13 ± 0.4 |
| 200 | 8 ± 1.0 |
| 4.8 | 235 ± 5.5 |
| 50 | 89 ± 4.5 |
| 200 | 54 ± 0.2 |

^a Mean of duplicate assays; one unit will release 1 μ mole paranitrophenol/min under conditions of assay.

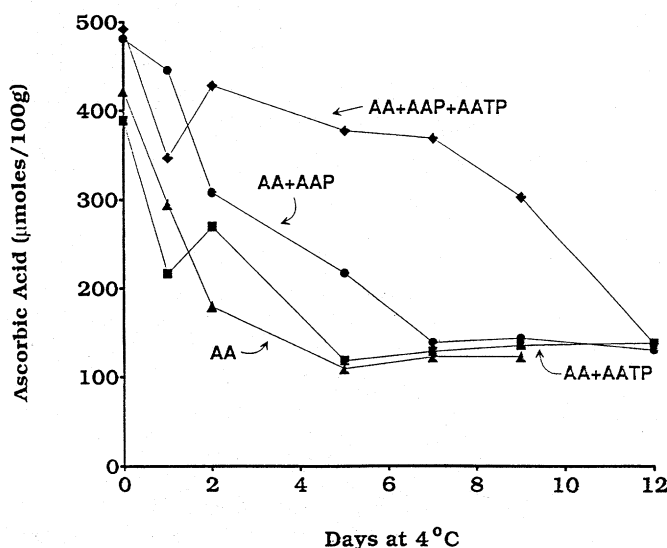


Fig. 1—Residual ascorbic acid (AA) in Russet Burbank plugs treated with combinations of AA, AA-2-phosphate (AAP), and AA-2-triphosphate (AATP) and stored at 4°C. AA = 2%; AAP (Na salt) = 3.7%; AATP = 6%; all dips contain 1% citric acid and 0.2% CaCl_2 ; pH adjusted to 2.1 with HCl.

viously (Sapers et al., 1990). Additional determinations for residual AA, AAP, ascorbic acid-2-diphosphate (AADP) and AATP in treated Russet dice were made by the HPLC method of Liao and Seib (1990), modified by the use of a 95:5 (v/v) mixture of acetate buffer (pH 4.7) and methanol containing 1.0 mM tetrabutylammonium phosphate and 0.2 mM EDTA as the mobile phase and by the omission of acetate buffer from the extraction solution and standards.

Effect of pH, phosphate and substrate on APase activity

APase activity was determined in Russet Burbank juice, adjusted to different pH values with citric acid and NaOH, and containing 0–200 mM sodium dihydrogen phosphate, by the colorimetric p-nitrophenol phosphate hydrolysis procedure (Sigma Procedure No. 104, Sigma Chemical Co., St. Louis, MO), as described previously (Sapers et al., 1991). Hydrolysis rates of AAP and AATP by APase were compared in a model system comprising 11.4 mmol AAP (Mg salt) or AATP (Na salt) in 100 mL pH 4.8 citrate buffer at 20°C, to which 3 mg (120 units) sweet potato APase (Sigma Chemical Co.) were added at zero time. AA in the reaction mixture was determined by HPLC, as described.

Weight loss in treated plugs

Russet plugs were treated by dipping for 5 min in a conventional dip or various experimental formulations. The treated plugs and untreated controls (dipped 20 sec in water) were weighed immediately

following treatment and after storage for as long as 13 days at 4°C to determine losses due to leakage or dehydration. The bottom surface of each plug was blotted on three layers of absorbent tissue prior to weighing to remove adhering droplets of liquid resulting from leakage.

Statistical analyses

Dipping trials and reflectance measurements for potato plugs were carried out with two or four replications. HPLC analyses for residual AA or AA-2-phosphates were performed in duplicate. Differences in browning inhibitor content or response to treatment were examined by the Bonferroni LSD mean separation test (Miller, 1972).

RESULTS & DISCUSSION

Control of APase activity

Initial attempts to improve the performance of browning inhibitors containing 1–2% AA and 1% CA by adding AAP or AATP at levels equivalent to 1% AA were not successful. Tristimulus measurements indicated that the inclusion of AA-2-phosphates in dips did not significantly improve the stability of treated Russet plugs, all samples failing within 2–3 days at 4°C. Based on our hypothesis that the effectiveness of AAP's in potatoes was limited by the high APase activity found in this commodity (Sapers et al., 1991), we investigated means of decreasing APase activity by modification of browning inhibitor formulations. Acidification of Russet Burbank juice with citric acid to pH 3.0 or 2.0 decreased APase activity by 74 and 98%, respectively, compared to the activity at pH 5.8, the original juice pH (Table 1). Addition of 200 mM phosphate to the juice at pH 3 and 4.8 decreased APase activity by 77 and 67%, respectively.

A further decrease in the rate of hydrolysis of ascorbic acid-2-phosphates could be obtained by substituting AATP for AAP. The hydrolysis of AAP and AATP by sweet potato APase in a model system at pH 4.8, measured by the appearance of AA, was linear with time at 20°C ($r = 0.99$). The hydrolysis rate of AATP, obtained by linear regression, was 0.27 μ moles ascorbic acid/mL/hr, compared to 2.5 for AAP. Liao and Seib (1990) also reported that AATP was hydrolyzed much more slowly than AAP; they used potato APase (Type IV-S, Sigma Chemical Co.) in a similar model system.

Development of AA-2-phosphate-based treatments

Based on those findings, we formulated dips containing combinations of AA, AAP and AATP together with CA and CaCl_2 , adjusted to pH 2.1 with HCl. Since the effectiveness of such a dip as a browning inhibitor would depend on the residual AA content in treated products, we measured residual AA in treated Russet Burbank plugs during storage at 4°C by HPLC (Fig. 1). Plugs treated with the AA-AAP combination retained AA longer than those treated with AA alone or the AA-AATP combination. The highest AA retention, however, was in plugs treated with the three-way combination of AA-AAP-AATP. The apparent low values on day 1 for residual AA in plugs treated with AA-AATP and the three-way combination were probably artifacts due to sampling. The three-way combination should provide initial protection against browning, via AA added prior to the accumulation of AA arising from AAP hydrolysis, and long-term protection due to the gradual hydrolysis of AATP following AAP depletion.

Comparison of the extent of browning in Russet plugs and dice, treated with experimental formulations based on combinations of AA, AAP and AATP that were acidified to pH 2 with H_3PO_4 vs. conventional formulations confirmed the efficacy of our experimental approach (Table 2). In this experiment, the dipping time was extended from 90 sec to 5 min since earlier studies (data not shown) indicated that this modification would increase AA uptake by about 8% and improve product storage life. The storage life of samples was extended by about 1 wk to 9 days for plugs and 13 days for dice by the

Table 2—Control of browning in Russet plugs and dice with experimental vs conventional formulations, applied by dipping for 5 min

| Type | Treatment ^a | Percent inhibition (a-value) | | | | |
|------|--------------------------------------|------------------------------|-----------------|-----------------|-----------------|-----------------|
| | | 2 | 5 | 7 | 9 | 12 |
| Plug | Conv | 72 | 24 ^c | 27 ^c | 21 ^b | 2 ^b |
| | Exp + HCl | 71 | 43 ^c | 40 ^c | 30 ^b | 16 ^b |
| | Exp + H ₃ PO ₄ | 79 | 78 ^b | 79 ^b | 58 ^b | 22 ^b |
| Dice | Conv | 3 | 6 | 8 | 10 | 13 |
| | Exp + HCl | 101 | 58 ^c | 17 ^c | 19 ^d | 6 ^d |
| | Exp + H ₃ PO ₄ | 91 | 90 ^b | 90 ^b | 61 ^c | 38 ^c |
| | | 100 | 99 ^b | 91 ^b | 88 ^b | 56 ^b |

^a Conv: 4% AA + 1% CA + 1% SAPP + 0.2% CaCl₂.

Exp: 2.5% AA + 1% CA + 1% SAPP + 0.2% CaCl₂ + 1.9% AAP (Na salt) + 1.5% AATP (Na salt) + HCl or H₃PO₄ to pH 2.0.

^{b-d} Mean of two (plugs) or four (dice) replicates; means within columns for each sample type, followed by different superscripts, are significantly different at P < 0.05 by the Bonferroni LSD test.

Table 3—Concentrations of ascorbic acid (AA), ascorbic acid-2-phosphate (AAP), ascorbic acid-2-diphosphate (AADP), and ascorbic acid-2-triphosphate (AATP) in treated Russet dice vs browning during storage at 4°C

| Day | % Inhibition (a-value) | | Concentration (μmole/100g) ^a | | | | |
|-----|---------------------------|---------------------------|-----------------------------------------|-----------------|-----|-------------------|------|
| | Conventional ^b | Experimental ^b | Conventional | Experimental | | | |
| | | | AA ^c | AA ^c | AAP | AADP ^d | AATP |
| 0 | 100 | 100 | 1495 | 1068 | 372 | 12 | 248 |
| 2 | 93.7 | 95.2 | 1440 | 923 | 186 | 129 | 50 |
| 5 | 79.4 | 96.4 | 909 | 1084 | 128 | 41 | 0 |
| 7 | 56.1 ^a | 93.1 | 585 | 1230 | 80 | 14 | 0 |
| 9 | 8.3 | 71.1 ^a | 235 | 746 | 10 | 11 | 0 |
| 12 | -20.2 | 24.5 | 200 | 492 | 2 | 14 | 0 |

^a Mean of duplicate analyses, performed on duplicate samples.

^b See Table 2, Footnote a; dips adjusted to pH 2.0 with H₃PO₄.

^c Endogenous AA = 36 μmoles/100g (63 ppm).

^d Tentatively identified.

^a Onset of browning, judged visually and by increase in a-value.

Table 4—Control of browning in high pressure steam-peeled Russet potatoes with experimental vs conventional formulations

| Treatment ^a | Percent inhibition (a-value) | | | | |
|------------------------|------------------------------|-----------------|-----------------|-----------------|-----------------|
| | 2 | 6 | 8 | 10 | 13 |
| Conventional | 97 ^b | 35 ^c | 14 ^c | -2 ^c | -6 ^c |
| Experimental | 102 ^b | 88 ^b | 75 ^b | 59 ^b | 44 ^b |

^a See Table 2, footnote a; dips adjusted to pH 2.0 with H₃PO₄.

^{b-c} Mean of four replicates; means within columns followed by different superscripts, are significantly different at P < 0.05 by the Bonferroni LSD test.

Table 5—Control of browning in abrasion-peeled Russet potatoes with experimental vs commercial formulation

| Days at 4°C | Treatment | Percent inhibition | |
|-------------|---------------------------|--------------------|-------------------|
| | | L-value | a-value |
| 4 | Commercial ^a | 70.9 ^d | 42.0 ^d |
| | Experimental ^b | 94.4 ^c | 83.8 ^c |
| 7 | Commercial | 64.8 ^d | 25.0 ^d |
| | Experimental | 90.8 ^c | 79.0 ^c |
| 9 | Commercial | 57.5 ^d | 15.8 ^d |
| | Experimental | 78.4 ^c | 68.8 ^c |
| 10 | Commercial | 58.4 ^d | 15.5 ^d |
| | Experimental | 79.6 ^c | 60.0 ^c |

^a Commercial, applied as directed by manufacturer.

^b See Table 2, footnote a; dip adjusted to pH 2.0 with H₃PO₄.

^{c-d} Mean of four replicates; means within columns for each day, followed by different superscripts, are significantly different at P < 0.05 by the Bonferroni LSD test.

experimental treatment. Dice responded better than plugs, presumably because of their greater surface to volume ratio which would allow them to absorb more browning inhibitor solution. The use of H₃PO₄ for pH adjustment proved more effective than adjustment with HCl, in spite of the fact that chlorides are well-known PPO inhibitors (Mayer and Harel, 1979). The level of phosphate in dips resulting from pH adjustment with

Table 6—Weight loss in plugs from high pressure steam-peeled potatoes treated with experimental and conventional browning inhibitor formulations

| Potato type | Treatment | Weight loss (%) | |
|-------------|-------------------------|-------------------|--------------------|
| | | Day 2 | Day 8 |
| Russet | Control | 0.72 ^c | 2.22 ^c |
| | Conventional | 1.18 ^c | 2.68 ^{bc} |
| | Exp with Na salt of AAP | 4.09 ^b | 3.85 ^b |
| | Exp with Mg salt of AAP | 3.75 ^b | 3.56 ^b |
| Round-white | Control | 1.04 ^d | 1.74 ^d |
| | Conventional | 1.43 ^d | 2.26 ^d |
| | Exp with Na salt of AAP | 6.58 ^b | 5.30 ^b |
| | Exp with Mg salt of AAP | 5.24 ^c | 3.64 ^c |

^a See Table 2, footnote a; dips adjusted to pH 2.0 with H₃PO₄; AAP (Mg salt) = 1.6%.

^{b-d} Mean of four replicates; means within columns for each potato type, followed by different superscripts, are significantly different at P < 0.05 by Bonferroni LSD test.

H₃PO₄ was about 350 mM, almost double the maximum concentration used in the APase inhibition experiment.

Both experimental and conventional dips contained 1% SAPP, used commercially as an inhibitor of after-cook darkening (Smith, 1987) and as a component of several sulfite substitutes (Duxbury, 1988; Santerre et al., 1991), and 0.2% CaCl₂. Calcium salts may be added to processed fruits and vegetables as firming agents. However, added Ca may also strengthen cell walls and membranes (McGuire and Kelman, 1984), resulting in less leakage of PPO or its substrates at cut surfaces.

The greater effectiveness of the experimental formulation over the conventional dip is probably due to the rate of hydrolysis of the AA-2-phosphates during storage and resultant accumulation of AA in treated samples. Concentrations of AA and the AA-2-phosphates in treated Russet dice, determined by the HPLC procedure of Liao and Seib (1990), were compared with stability data (Table 3). Immediately after treatment, the dice contained 1495 and 1068 μmoles AA/100g (2633 and 1880 ppm) for conventional and experimental formulations, respectively. During storage, the AATP concentration decreased rapidly while, at the same time, a new peak appeared, tentatively identified as ascorbic acid-2-diphosphate (AADP), based on the chromatographic data of Liao and Seib (1990). That peak in turn disappeared, presumably undergoing further hydrolysis to AAP. The AAP content of treated dice decreased more slowly, acting as a reservoir for the gradual release of AA. Consequently, the AA content of dice treated with the experimental formulation exceeded that of conventionally treated dice on day 5 and remained high enough to delay browning until day 9 when slight browning was observed and the AA content decreased to 746 μmole/100g (about 1300 ppm). In contrast, the conventionally treated dice became noticeably discolored on day 5 when the AA content was 909 μmole/100g (about 1600 ppm) and slightly brown on day 7 when the AA content decreased to 585 μmole/100g (about 1000 ppm).

Application of treatments to pre-peeled potatoes

The experimental treatment was also more effective than the conventional dip when applied to peeled potatoes. Controlling browning at the peeled surface of potato is much more difficult than preventing browning in plugs or dice because of the extensive tissue damage caused by the peeling process. Nevertheless, in high pressure steam-peeled Russet potatoes browning was delayed by the experimental treatment, resulting in a storage life of 8–10 days, compared to less than 6 days for the conventionally treated sample (Table 4). Similar results were obtained with round-white potatoes (data not shown).

The experimental formulation also was compared with a commercial browning inhibitor, containing the same ingredients as our conventional dip and applied as directed by the manufacturer, in trials with abrasion peeled Russet potatoes (Table 5). The experimental treatment resulted in a storage life

of 9-10 days, compared to less than 4 days for the commercial treatment, based on the change in L- and a-values.

During storage of potato plugs and dice treated with experimental formulations, some leakage was observed within the first few days following treatment, resulting in a weight loss of 4-6%, the loss being greater in round-white than in Russet type potatoes (Table 6). In contrast, conventionally treated samples and controls showed a gradual weight loss of about 2% during the same period, without visible leakage. The loss in samples treated with the experimental formulations was reduced slightly by substitution of the magnesium salt of AAP for the sodium salt. Other modifications in the formulation (0.2% additional CaCl_2 , 1% additional citric acid, no citric acid in dip) were without effect. Leakage induced by the experimental treatment probably is an osmotic effect resulting from phosphate addition during pH adjustment. Such leakage did not appear to affect sample storage life. However, further research is needed to determine whether leakage would result in handling problems or adversely affect product quality. Further research also is required to establish whether the application of highly acidic browning inhibitor formulations to pre-peeled potatoes or dice would affect the flavor of cooked products.

CONCLUSIONS

THE EFFECTIVENESS of AA-based browning inhibitor formulations for potatoes was improved greatly by replacing some of the AA with AA-2-phosphates. To obtain extended control of browning in diced and pre-peeled potatoes, such formulations should contain both AAP and AATP and should be acidified to pH 2 with H_3PO_4 . Before such browning inhibitor formulation could be considered for commercial use, however, the problem of leakage induced by the treatments, the sensory attributes of treated potatoes, and the economic feasibility should be addressed, and FDA approval for use of AA-2-phosphates in foods must be obtained.

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